Supplemental Figures

Supplemental Figure 1: Influence of Vinculin on hMSCs. A) Western blots for vinculin and GAPDH are shown for cells treated with siRNA for vinculin over the indicated time course. The plot quantifies vinculin knock down after 24 hours of approximately 80% when normalized to GAPDH band intensity at each time point or condition. Characteristic recovery was observed over one week. Transfection efficiency was confirmed using a fluorescent siGLO oligomer (inset). B) MyoD stained images of non-targeting control siRNA (scrambled) did not show a significant difference in MyoD expression versus wild type (WT) as quantified by fluorescent intensity. C) MyoD stained images of vinculin knockdown cells cultured for 4 and 12 days recover MyoD expression only after 12 days in culture. *$p < 0.05$, ****$p < 0.0001$, N.S. = not significant. Scale Bars: B, C = 10 μm,

Supplemental Figure 2: Vinculin Knockdown and Osteogenic Differentiation. hMSCs on 34 kPa matrices acquired expression of CBFA1, an

osteogenic marker, between days 2 and 4 in culture. Vinculin knockdown did not affect the expression of A) CBFα1 or B) ALP. Scale Bars: A = 10 μm, B = 50 μm.

Supplemental Figure 3: Cell Migration in Knockdown Cells. A) Average hMSC velocity was measured for 24 hours post-plating. Cell velocities were found to be statistically similar regardless of vinculin siRNA treatment. B) Rose plots show no preferential migration patterns of wild type or vinculin knockdown cells on static 1 kPa and 11 kPa hydrogels.

Supplemental Figure 4: Add-back. A) qPCR was used to assess uptake and expression of chicken vinculin constructs. For each primer, the corresponding sample was used to normalize its expression, e.g. vinculin tail added cells were used to normalize data using the tail primer. Endogenous mRNA was found in wild type cells and significantly reduced in all knock down samples. The head primer was amplified in the head and full length add back samples, while the tail primer was amplified in the tail and full length add back samples. A full length primer spanning multiple domains was only amplified in the full length add back sample. *$p < 0.05$, **$p < 0.01$ versus WT as indicated by ANOVA. B) Sanger sequencing results confirmed the change in nucleotide sequence (arrowheads) in FL-Mut samples as a result of mutagenesis.

Supplemental Figure 5: MyoD and MAPK1 inhibition. A) Immunofluorescent images of hMSCs treated with 5-iodotubercidin, pyrazolylpyrrole, and/or vinculin

siRNA (Vinc KD) as indicated on 11 kPa matrices and stained for actin, nuclei (DAPI), and MyoD. B) Quantification of vinculin intensity in hMSCs cultured under the indicated conditions. ****$p < 0.0001$ as indicated by ANOVA. C) Immunofluorescent images for vinculin of wild type hMSCs or hMSCs treated with 5-iodotubercidin and pyrazolylpyrrole. Arrowheads indicate focal adhesions. D) Cell area of wild type hMSCs and hMSCs treated with the indicated combinations of 5-iodotubercidin, pyrazolylpyrrole, and vinculin siRNA. No comparisons were statistically significant. E) hMSCs were cultured with or without pyrazolylpyrrole and/or vinculin siRNA, and the percentage of hMSCs on stiff regions of a matrix with alternating stiff and soft stripes was quantified. Data was collected shortly after plating (1 hr) and 24 hrs post-plating. ****$p < 0.0001$ as indicated by ANOVA. Scale Bars: A, C = 20 μm.

Supplemental Figure 6: Vinculin knockdown does not affect proliferation or focal adhesion dynamics. A) Low magnification images of wild type (WT) and siRNA-induced vinculin knockdown (KD) hMSCs after 0, 2, and 4 days of culture stained for actin to illustrate cell density over time. Vinculin knockdown's affect on B) cell area (gray) and aspect ratio (the ratio of the major to minor cell axis; white), C) focal adhesion number per cell as measured from Paxillin stained cells, and D) total focal adhesion area as measured from Paxillin stained cells. E) Live (green)/dead (red) assay of cells with the indicated treatments. Scale Bar: A = 2 mm, E = 50 μm. N.S. = not significant.

Supplemental Figure 7: Radial Shear Assay. A) To test adhesion strength of our cells, a spinning disc assay was utilized. Cells were plated onto round coverslips and subjected to circular motion, resulting in a shear force over the cells that is proportional to their distance from the center of the coverslip. B) Binned cell density is plotted against shear force and fitted by a sigmoid function (red line). The average adhesion strength (τ_{50}) is defined by the shear required to detach 50% of the cells (dashed lines). C) Representative images for the center (i), middle (ii), and edge (iii) of the cover slips are shown with DAPI stained nuclei. Scale Bar: A = 50 μm.

Supplemental Figure 8: Traction Force Microscopy in hMSCs. To assess the ability of a cell to generate strain energy against its substrate, Traction Force Microscopy was utilized. Matrix deformation fields (A) can be resolved into tangential (B) and normal (C) stress maps. The tangential and normal strains can then be integrated over each cell to find the work done by the cell to deform the matrix in either direction (D). Scale Bar: A = 20 μm.

Supplemental Tables

Supplemental Table 1: Surface accessibility values for 49 human focal adhesion proteins. Accessibility was determined by ScanSite analysis (Obenauer et al, 2003) where surface accessible and inaccessible kinase binding sites have values above 1 and below 1, respectively. ScanSite provides a single accessibility value based on the amino acid sequence surrounding each point.

Kinase abbreviations: c-abl oncogene 1, non-receptor tyrosine kinase, ABL1; RAC-alpha serine/threonine-protein kinase, AKT1; Amphiphysin, AMPH; Ataxia telangiectasia mutated, ATM; calcium/calmodulin-dependent protein kinase II gamma, CAMK2G; Cyclin-dependent kinase 1/Cell division control protein 2, CDC2; Cyclin-dependent kinase 5, CDK5; CDC-like kinase 2, CLK2; p38-CRK sarcoma virus CT10 oncogene homolog, CRK; Casein kinase 1 gamma 2, CSNK1G2; Casein kinase 2 beta polypeptide, CSNK2B; Receptor tyrosine-protein kinase erbB-1, EGFR; p55-FGR, FGR; p59-FYN, FYN; Growth factor receptor-bound protein 2, GRB2; Glycogen synthase kinase 3 alpha, GSK3A; Glycogen synthase kinase 3 beta, GSK3B; Hematopoietic cell-specific Lyn substrate 1, HCLS1; Inositol polyphosphate-5-phosphatase, INPP5D; CD220, INSR; IL2-inducible T-cell kinase, ITK; Intersectin, ITSN; Lymphocyte-specific protein tyrosine kinase, LCK; Mitogen-activated protein kinase 1, MAPK1; Mitogen-activated protein kinase 3, MAPK3; Non-catalytic region of tyrosine kinase 1, NCK1; Platelet-derived growth factor receptor beta, PDGFRB; Phosphoinositide dependent protein kinase-1, PDPK1; Phosphoinositide-3-kinase, regulatory subunit 1, PIK3R1; Phosphoinositide-binding protein, PIP3-E; Phospholipase C gamma 1, PLCG1; Protein kinase, AMP-activated alpha 1, PRKAA1; Protein kinase cAMP-dependent catalytic delta PRKACD; Protein kinase cAMP-dependent catalytic gamma, PRKACG; Protein kinase C alpha, PRKCA; Protein kinase C delta, PRKCD; Protein kinase C epsilon, PRKCE; Protein kinase C mu, PRKCM; Protein kinase C zeta, PRKCZ; Protein kinase, DNA-activated, catalytic polypeptide, PRKDC; Src homology 2 domain containing

protein 1, SHC1; Sorbin domain containing 1, SORBS1; c-Src, SRC; 14-3-3 Mode 1, YWHAZ

Supplemental Table 2: Human qPCR primers. Below is a list of primers used to amplify sample cDNA.

Supplemental References

Flores GV, Duan H, Yan H, Nagaraj R, Fu W, Zou Y, Noll M, Banerjee U (2000) Combinatorial signaling in the specification of unique cell fates. *Cell* **103:** 75-85

Obenauer JC, Cantley LC, Yaffe MB (2003) Scansite 2.0: Proteome-wide prediction of cell signaling interactions using short sequence motifs. *Nucleic Acids Res* **31:** 3635-3641